There is also the point whether the colour of the sides of the mess-deck has any effect upon the *feeling* of warmth or of coolness, even though this may operate through suggestion only. It has been the tradition to paint the interior of sick-bays a pale green in tropical-going ships. F. C. Houghton, H. T. Olson, and J. Suciu found no objective change in skin temperatures in different coloured environments, but they affirmed that occupants might experience a subjective sense of warmth or of coolness in the presence of this colour or that—which, from the human standpoint, is significant.

Body-cooling in hot atmospheres may be obtained by adequate draughts of cold water ; in any event the need for drinking, over and above the call of thirst alone, is important for maintaining mental and physical efficiency in high temperatures. There is a rationale, therefore, for supplying cooled drinking-water in working- and living-spaces, though the American habit of iced water may not be a physiological ideal. Whether the water should contain salt or oatmeal needs closer investigation.

Lastly, the innovation of laundries in H.M. Ships has much to recommend it, not only because they promote cleanliness (and so avert skin infection) and avoid the wearing of sweaty garments (which aggravates prickly heat), but chiefly because they abolish wet garments from the mess-decks. There is the point, too, that sweat-soaked garments will not readily dry except in an atmosphere of extreme desiccation—a condition which does not obtain in the mess-decks.

(These lectures will be continued in the issue of Aug. 11 and completed in that of Aug. 18)

BACTERICIDAL EFFECT OF MIXTURES OF ETHYL ALCOHOL AND WATER

WITH SPECIAL REFERENCE TO STERILIZATION OF THE SKIN, AND A NOTE ON THE COMPARABLE EFFECTS OF ETHER

BY

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Although much work has been done by a number of observers on the antibacterial effects of alcohol on the one hand and on the value of various substances as sterilizing agents for use on the skin on the other, agreement would appear not to have been reached regarding the optimal dilution of alcohol for use on the skin before puncture or surgical incision. Precision on two points is necessary for a final assessment : first, percentages of alcohol in water should be accurately defined in respect of any observations made ; and, secondly, the requirements of a fluid for use on the skin should be considered in terms of its physical properties as well as its bactericidal power. Further, due consideration must be given to the bacterial species which it is most necessary to eliminate.

Percentages of Alcohol

For scientific accuracy it is probably desirable that the strength of an alcohol mixture should be recorded as percentage by weight. On the other hand, under ordinary conditions the preparation of volumetric percentages can more readily be carried out. There are appreciable differences in the strength of mixtures produced by (*a*) making up a measured volume of alcohol to a total volume by the addition of water ; (*b*) making up a measured volume of water to a total volume by the addition of alcohol ; and (*c*) mixed measured volumes of the two fluids. These differences are due to concentration during admixture. The *first* is the correct percentage by volume. The following figures, calculated from tables given in *The Chemists' Year Book, 1942*, illustrate the differences in strength between solutions made up by the methods referred to :

(1) 76% by weight is approximately equal to 82% by volume, at 60° F.
(2) 70% " " 77% " "
(3) A mixture made by making up 30 parts of water to 100 parts by the addition of alcohol is approximately equal to 73% by volume.
(4) A mixture made by the addition of 30 parts of water and 70 parts of alcohol is approximately equal to 72.5% by volume.

Physical Properties of Alcohol-and-Water Mixtures

From a practical point of view *surface tension* is probably among the most important variable characteristics of alcohol-and-water mixtures. It is likely that low surface tension is the factor mainly responsible for the improved spread (Bonney and Allen, 1944), penetration (Lovell, 1945), and complete wetting observed when alcohol is used on the skin. The variation in surface tension which occurs as alcohol is progressively diluted with water is illustrated by the following experiments. It was also apparent in some of the experiments on plates of solid media referred to later in this paper.

Experiment A

Mixtures of water and alcohol of 65 volumes of alcohol and 35 volumes of water, and of 30 volumes alcohol and 70 volumes of water respectively, were dropped from a Dreyer's pipette on to a porcelain tile which had been rubbed over with Congo-red powder. The diameters of the circles of spread of the alcohol were measured and the volume of the drops calculated by counting the drops in 0.5 c.cm. The mean diameter of the area wetted by the drop of the stronger mixture was 2.7 cm. (mean of 10 observations), while the mean volume of the drop was 16.6 c.mm. The figures for the weaker alcoholic mixture were 2.05 cm. (mean of 10 observations) and 19.23 c.mm. respectively.

Experiment B

A somewhat more careful observation, using absolute alcohol and a mixture of 65 volumes of alcohol and 35 volumes of water respectively, was made by tracing the outline of the wetted areas produced on a similarly prepared tile, measuring the areas enclosed, and calculating the diameter. The drop volumes were obtained by measuring the volume of 200 drops of each strength of alcohol. In this experiment the mean diameter of the area wetted by 16.375 c.mm. drops of absolute alcohol was 3.37 cm., while that of the area wetted by 17.625 c.mm. drops of " 65% " alcohol was 2.85 cm.

Species of Pathogenic Bacteria Liable to be Found on the Skin

Bacteria encountered on the skin may be divided into " resident " and " transient " organisms. The former cannot be completely eliminated by any process which will not injure the tissues (Colebrook, 1941). Much of the " resident flora " is located in the pilonidal follicles ; these are not penetrated by 70% alcohol or by acetone (Lovell, 1945). According to Colebrook (1941) *Str. pyogenes, Proteus,* and *Ps. pyocyanea* are among the transient flora, and *Staph. pyogenes* is difficult to place, being intermediate between the transient and resident flora. He quotes various workers as finding *Staph. aureus* (*pyogenes*) on the skin of from 5% to 30% of normal individuals. Martin (1942) reports the occurrence of *Staph. aureus* on the face, wrist, and chest respectively in 38%, 24%, and 14% of normal individuals, the corresponding figures of *Str. pyogenes* being 5%, 3%, and 1%. Miles *et al.* (1944) report the skin carriage of *Staph. aureus* in 18.4% of individuals. Cruickshank *et al.* (1942) quote Lockemann *et al.* (1941) as finding *Staph. aureus* more resistant to all strengths of alcohol than either *Bact. coli* or *Ps. pyocyanea.*

It would thus seem that the power to kill *Staph. aureus* may be considered as a suitable index of the value of alcohol in skin sterilization. It should be noted, however, that, owing to the alcohol-fast nature of the genus *Mycobacterium,* alcohol may be expected to be inefficient as a bactericidal fluid for *M. tuberculosis.* It is possible, though hardly probable, that failure in this respect led to an accident following which the use of alcohol for sterilizing syringes for the administration of parenteral injections was condemned by Bigger *et al.* (1940), though these workers also showed that alcohol failed to destroy streptococci in sputum used to contaminate syringes in certain of their experiments.

Recommendations by Previous Observers regarding Suitable Strength of Alcohol

Where contact may be prolonged weak mixtures are effective. Cruickshank *et al.* (1942) have shown that *Staph. aureus, Bact. coli,* and *Ps. pyocyanea* may be destroyed in from 0.5 to 24 hours by 25% alcohol. The length of time necessary to achieve sterility in certain of their experiments shows that 25% is obviously an ineffective strength for use in skin sterilization, where contact of the reagent with the skin is limited by the volatile nature of alcohol and by considerations of practical

utility. These authors quote Minervini (1898), however, as having shown that the most germicidal concentration of alcohol is between 50% and 70%. Garrod and Keynes (1937) state that the optimum strength of alcohol is 70%. Bigger *et al.* (1940), while condemning the use of alcohol for sterilizing syringes as stated above, consider that a solution containing 70% alcohol has a much greater bactericidal action than the 95% spirit described as suitable by Lawrence (1940). The *British Medical Journal* (1940, **1**, 96) in a leading article quotes Regamey (1939) as finding that *Bact. coli* in broth culture was destroyed within 15 seconds by all concentrations of alcohol between 50% and 99%, and within one minute by 40%. *Silk threads* contaminated with *Bact. coli*, however, were sterilized in one minute only by concentrations of 50% and 60%; 70% took 3 minutes, 80% 15 minutes, while in 90% and 99% the organisms survived for 24 hours. Similar results were found with staphylococcus and streptococcus, except that the optimum concentration for staphylococcus was somewhat higher.

For use *on the skin* the following recommendations have been made from time to time:

95%: Hatfield and Lockwood (1943) found that the best results were obtained when 95% alcohol was used after *tincture of iodine*, but that consistently low counts were obtained after alcohol alone.

76% by weight: Colebrook (1941) reports the use of alcohol of this strength against streptococci on the skin—the streptococci were not completely eliminated.

70% by weight: Price (1939) is quoted by Colebrook as having shown that this is the most effective concentration of alcohol.

70% by volume: Has been recommended in certain official publications.

Various references to "70% spirit," "75% spirit," etc., may be found in the medical press and in commercial medical literature.

In the course of some unpublished work on skin disinfection (using *Staph. aureus* as an "indicator organism") carried out in 1941 by B. W. Lacey and myself, "industrial spirit" was used as a control in all experiments. This was very effective, but it was decided for the sake of completeness to carry out an *in vitro* test of the relative value of falling dilutions of alcohol. Method 1 of those given below was used, and results suggested that 65% was below the effective level for a period of exposure up to two minutes. 70% was not used.

Ether.—Ether is commonly applied to the skin as a part of the pre-operation toilet. It was found ineffective as a fluid for sterilizing syringes by Bigger *et al.* (1940), and its use as one of the reagents tested in the above-mentioned work by Lacey and myself suggested that its bactericidal effect when applied to the skin is inconsiderable.

Present Investigations

Purpose.—In view of the facts and considerations referred to above it was thought worth while to test yet again the *in vitro* effect of alcohol-water mixtures on *Staph. aureus* over a narrow time-range; to test the effect of such dilutions on the artificially contaminated skin; to compare the results obtained by different technical methods, and with different organisms; and to carry out a small series of parallel tests with ether. In all of the tests, unless otherwise stated, the dilutions used were made by mixing *measured volumes* of alcohol and water. They were thus slightly stronger than their nominal strength expressed as percentages by volume (see "Percentages of Alcohol" above).

Methods of Demonstrating Antibacterial Action Employed

1. *Glass Bead Method.*—Small unperforated glass beads as used in the "transfusion-giving set, hospital pattern," were placed in a thick suspension of organisms. They were then removed and dried. The dilutions of alcohol under test were poured on to sterile dishes, and a bead was placed in the mixture for a measured time; it was then transferred, with or without a preliminary washing in water, to sterile broth or to the surface of the agar in a Petri dish or covering one side of a 1-oz. screw-cap bottle. (When beads were transferred to the solid medium they were rolled freely about on the surface of the agar before incubation.) The media so inoculated were then incubated. A somewhat similar method—"Krönig and Paul's garnet method"—was employed by Hailer and Bockelberg (1939) in estimating the germicidal efficiency of the halogens on anthrax spores. In the course of the work it was found that washing of the alcohol-treated bead is necessary when it is to be used to inoculate a solid medium.

2. *Filter-paper Method.*—Small pieces of sterile filter-paper were immersed in a suspension of the organisms to be employed. After drying, these were placed in the spirit mixtures under test and after a noted interval transferred to bottles of broth, which were then incubated; or they were washed in water and placed on the surface of an agar plate, from which they were removed after a short time in the incubator, incubation of the plates being continued.

3. *Fluid Inoculum Method.*—A known volume of bacterial suspension was added to a known volume of the alcoholic mixtures under test. Drops of the resultant suspensions after shaking were then transferred, after an observed time of contact, to bottles of broth, which were subsequently plated and the plates incubated.

4. *Contaminated-finger Method.*—The fingers were contaminated by rubbing the tips over the surface of a plate culture. Individual fingers were then either (*a*) wiped with dry cotton-wool to spread the organisms further and remove excess, after which they were dipped for a moment in dilutions of alcohol; or (*b*) they were wiped with cotton-wool moistened with the alcohol dilutions. The fingers were then left until apparently dry, after which they were again wiped on fresh plates of a similar medium.

5. *Prevention of Growth on Inoculated Solid Media.*—Areas of plates of appropriate media were inoculated with the indicator organisms. When these areas were dry drops of the test dilutions of alcohol were delivered to the middle of each area with a Dreyer's pipette and the plates incubated when again dry. The use of blood-agar plates and *Str. pyogenes* as the test organism afforded an excellent "indicator" method, as the effective concentrations were made very obvious by the failure of haemolysis in the area of spread of the alcoholic drops. To exclude the possibility that failure of growth after the application of alcohol to an inoculated area might be due to destruction of the nutrient properties of the medium (Hayward and Miles, 1943; Cawston and Colebrook, 1943) rather than to death of the organisms, the following controls were incorporated in two of the experiments carried out on *Str. pyogenes* by this method.

Control A

The centres of the areas covered by the spread of the drops of various dilutions of alcohol used—from 100% to 40%—were reinoculated with the strain under test after the alcohol had dried. Growth occurred as a result of each such reinoculation.

Control B

Parts of the areas over which each alcoholic dilution had spread were cut out and placed, inoculated surface down, upon a fresh plate of blood agar. *Growth failed to occur* where contact was made between the fresh plate and the alcohol-treated part of the excised portion of medium. *Growth occurred* where the fresh plate was in contact with such an area of the cut-out piece as had not been covered by the alcohol.

Hence (*a*) the medium was not destroyed by such exposure to alcohol as the method entailed, (*b*) the organisms were killed by the alcohol in the strengths which led to failure of growth.

6. *Killing of a dried film of organisms on a slide by the application of drops of descending dilutions of alcohol;* as indicated by the failure of growth development when the treated slide is applied to the surface of a plate of medium and incubated.

Results

Method 1.—The results of four experiments using *Staph. pyogenes* showed that for periods of contact ranging from 5 seconds to 2 minutes 100% and 98% alcohol are highly ineffective and 95% is less bactericidal than mixtures containing more water. The effect of 50% alcohol is probably less than that of mixtures containing less water. The optimum range therefore would appear to be between 60% and 90%. Two experiments indicate that 60% and stronger mixtures of methyl alcohol are as effective as dilutions of ethyl alcohol in killing *Staph. pyogenes*. Single tests suggested that "industrial spirit" and methylated spirit (tinted violet) are effective against *Staph. pyogenes* in a range from full strength to 60% or weaker.

The results of these experiments do not confirm the failure to sterilize staphylococcus-contaminated beads by the use of 65% spirit found in the experiment carried out in 1941 and referred to above. The failure then observed may have been due to a technical error such as incorrect dilution of spirit or contamination during manipulation or to a fault inherent in the method, such as bubble formation or clumping of the organisms with fixation of the outer layer and lack of penetration to the middle of the clump. It is considered that bubble formation is a likely cause, since this would tend to become more frequent as surface tension rises with increasing dilutions of the alcohol. It was thought to be the cause of a completely irregular result when the method was tried with a watery disinfectant. Similar irregular results, though to a lesser degree, have occurred throughout the experiments here summarized. They rendered the method inaccurate, particularly in fluid media. It was therefore discontinued.

Method 2:—Two experiments were made using this method on *Staph. pyogenes*. The method was not found very satisfactory. The first experiment, in which the pieces of filter-paper were placed in broth alone, after 10 seconds' treatment in each case, produced only two sterile cultures—one after treatment with 95% alcohol, the other after 65% alcohol. With reference to the latter, however, it was realized at the time that, owing to a hitch, exposure had exceeded 10 seconds; the test with this dilution had been repeated, and in the second broth growth occurred. The second experiment indicated that absolute alcohol is inefficient, while 80% to 50% were all bactericidal in 30 seconds, as shown by sterile results of both fluid and plate culture. Culture after 10 seconds suggested that 80% was less effective than the weaker mixtures, of which 60% gave the best result.

Method 3.—Using *Staph. pyogenes* and *Str. pyogenes*, one experiment for each was carried out. In the case of the test on *Staph. pyogenes* there was no growth on subcultures from mixtures containing 95% to 47.5% of alcohol after one minute's contact. A heavy growth occurred on subculture of a mixture containing 30% of alcohol after a similar time, but this strength was bactericidal after 15 minutes' contact. *Str. pyogenes* subcultures from mixtures containing dilutions of alcohol from 95% to 38% showed no growth after 10, 30, and 60 seconds' contact.

In view of these findings it seemed desirable to make further observations on the artificially contaminated skin. Six such tests were made as shown under Method 4.

Method 4.—Four experiments using *Str. pyogenes* were carried out. Two of these were partially spoiled by contamination. The combined results, however, indicate that, though of considerable value, absolute alcohol, and probably 70% alcohol, are less effective than mixtures of intermediate strength, while 65% showed a definite comparative inefficiency. Two of these experiments were made with true volumetric percentage dilutions. Two similar series of tests, also using correct volumetric percentage mixtures, were made on fingers contaminated with *Staph. pyogenes*. 100% to 65% were apparently effective; 60% was not.

In view of the difference between the results obtained with Method 4 and those which followed the use of the first three methods it seemed desirable to compare these findings with the effects of similar strengths of alcohol on inoculated areas on a plate of solid medium—i.e., on a surface upon which, like the skin, a certain dilution effect due to absorbable water is liable to occur. (It should be mentioned that all this work was carried out in a tropical climate of high humidity. The skin was thus liable to be more moist than is normal in a temperate or cold climate.)

Method 5.—Three experiments on cultures of *Staph. pyogenes* (Oxford) were made. The third test was carried out in duplicate, and true percentage dilutions by volume were used. In all experiments the best result was observed with strengths above 80% (*including absolute alcohol*); deterioration in antibacterial effect was marked by the time a dilution of 65% was reached. Eight experiments were carried out on cultures of *Str. pyogenes*. In four experiments true volumetric dilutions were used. The results of these tests may be summarized as follows:

A complete failure of growth, though over a diminishing area, resulted when alcohol of strength varying from 100% to 70% was dropped on plates inoculated with *Str. pyogenes*. An almost equally good effect was generally produced by 65% alcohol: 50% alcohol and weaker mixtures were relatively ineffective. The following chance observation is of interest. In one experiment the streptococcal culture used as an inoculum became contaminated with an organism giving a lemon-yellow colony on agar. Colonies of this contaminant appeared in considerable numbers in areas inoculated for the test and, while in this test the use of both 60% and 50% alcohol prevented the development of streptococcal colonies, scanty lemon-yellow colonies appeared on the area treated with 60% alcohol and growth of the contaminant was abundant on that treated with 50% alcohol.

In general it would appear (though this was not apparent in experiments using Method 4) that streptococci are killed by somewhat weaker alcohol than is necessary to kill staphylococci under parallel test conditions. One experiment was carried out on a local strain of *Ps. pyocyanea*: 100% to 60% were effective, 50% was not.

Method 6.—The discrepancy between the effects of spirit dilutions on dried inocula on glass (Method 1) and on inocula on the skin or on solid culture media (Methods 4 and 5) is thus considerable. The similarity between the observations recorded of the effects produced on the skin and on inoculated plates is equally striking. A final series of experiments was made to determine the effect on dry contaminated glass slides of drops applied in the manner previously used for inoculated solid media. The test thus resembled that on media in that the exposure to the reagent was determined by the time taken for the spirit to evaporate (rather than any fixed time interval), but differed in that the reduction of the alcohol ratio by absorption of moisture is avoided. Two experiments with *Str. pyogenes* and one each with *Staph. pyogenes* and *Ps. pyocyanea* were made. The two tests on streptococcus showed an effective strength down to 50%, with a limited effect produced by 40%. The experiment with staphylococcus showed an effective strength down to 60%: 50% was only slightly less effective; 40% also produced a limited effect The experiment with *Ps. pyocyanea* showed that 50% alcohol was effective; lower dilutions were not tested on this species.

The results therefore are more comparable with those found by Methods 1–3 than those following the use of Methods 4 and 5, *except that absolute alcohol appears to be effective when used as in Method 6*. This may be due to absorption of enough moisture from the damp air, contact with which is unavoidable in experiments carried out by this method, and hence absorption of moisture may not, in fact, be entirely eliminated.

Ether

In this series of tests Methods 3 (modified), 4, and 5 were used.

Ether Added to a Fluid Culture.—Single experiments on *Staph. pyogenes, Str. pyogenes*, and *Bact. typhosum* were made. Ether was added to broth cultures of staphylococci and *Bact. typhosum*. The cultures were shaken up with the ether, and one drop from a Dreyer's pipette was delivered on to a plate. The cultures were then left for approximately 5 minutes, re-mixed with ether by drawing up into the pipette and expelling again a few times, and again plated. Finally this was repeated after leaving the cultures for a further 15 minutes approximately. Control cultures before the addition of ether showed that both cultures produced a confluent growth on a plate, on the area where one drop from a Dreyer's pipette had spread. The results after treatment with ether were as follows:—*Staph. pyogenes:* First subculture after shaking with ether gave *confluent growth*. Subcultures after approximately 5 minutes gave an average of 14 colonies; there was no growth from the last subculture (approximately 20 minutes after first mixing with ether). There was no growth from *any* of the three subcultures of *Bact. typhosum*. A similar test with two local strains of *Str. pyogenes* showed no growth from subcultures carried out at once and after 5 minutes.

Method 4.—One experiment on *Staph. pyogenes* and one on *Str. pyogenes* were carried out. Ether was quite ineffective in destroying either staphylococcus or streptococcus on the skin.

Method 5.—The method was slightly modified by using increasing numbers of drops on a single spot on inoculated plates to observe if any poor result following single drops could be attributed to the very high rate of evaporation. Tests were made on cultures of *Staph. pyogenes, Str. pyogenes*, and *Bact. typhosum*, with the following results:—*Staphylococcus and streptococcus:* Drops of ether from 1 to 8 produced only a slight progressive thinning of growth in the area on which they fell. In an experiment on *Str. pyogenes* (Milne) accidental contamination again afforded interesting information. On this occasion a Gram-positive bacillus contained in the inoculum was absent from all areas where ether had fallen—though present on the surrounding inoculated area—in tests made with 1, 3, 6, and 8 drops of ether. *Bact. typhosum:* No growth of this organism occurred over areas covered by single or multiple drops of ether on an inoculated area on a plate.

Summary and Conclusions

Some 30 experiments, using six different methods, were carried out to test the effect of various dilutions of ethyl alcohol

on bacteria, with special reference to the optimal dilution for use on the skin. Observations were mainly made on *Staph. pyogenes* (usually the Oxford strain) and *Str. pyogenes* (Milne strain and local strains), but two tests were performed using a strain of *Ps. pyocyanea*. Six experiments, employing three methods, were made to observe the effect of ether on bacteria.

The methods used are described, and the result of the employment of each is summarized.

The following conclusions were drawn:

1. *Effective Strength of Alcohol for Destruction of Bacteria after Short Exposure.*—(*a*) *On a dry surface*:—The effective range of strengths of alcohol for the killing of non-sporing bacteria is between 90% and 50%. 95% and above are partially ineffective, 100% being markedly so. The lower surface tension of stronger alcoholic mixtures suggests that the upper limits of this effective range may be preferable to the lower, though against this must be considered the more pronounced fixing effect of strong alcohol, which may cause the coagulation of an exudate and the consequent protection of living organisms within the coagulum so formed (Bigger *et al.*, 1940). (*b*) *On the skin*:—Since the normal skin is more or less moist the effective range of alcohol for use upon it is somewhat different. 100% is commonly effective—at least on moister skins and under tropical conditions of temperature and humidity; while under similar conditions 60% to 65% may show a certain loss of efficiency. The washing of the skin before the application of alcohol might be expected to exert a similar effect unless subsequent drying were thorough. Further, the value of a low surface tension may well be of importance in increasing spread and penetration, for skin sterilization. It is therefore considered that, as a general recommendation under all climatic conditions, *80% of alcohol by volume* is probably most suitable for skin sterilization, though this will not be more effective than any other non-persistent agent for dealing with deep-lying resident flora.

2. *Bactericidal Effect of Ether.*—Ether is quite ineffective as a sterilizing agent for the skin, since its effect is very slight on staphylococci and streptococci when applied to a surface—though it is effective against certain bacillary forms, both Gram-positive and Gram-negative.

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The report for 1944 of the St. John Ophthalmic Hospital at Jerusalem has been issued from the Chancery of the Order, St. John's Gate, London, E.C. The hospital was founded in 1883, and during the year under review Field-Marshal Viscount Gort, High Commissioner of Palestine and Transjordan, paid a visit as a compliment to the work of the Order of St. John in Palestine. All the difficulties of perpetual changes of personnel have continued. So far as the decline in the number of patients is concerned, the Warden, Dr. Norman Manson, states that it is due to the impossibility of getting nurses and ward maids. This shortage is so acute in Palestine that several hospitals are almost closed. The improvement in the military situation has not been followed by any relief on the economic front; the cost of living is at least as high now as it was when Palestine was packed with troops. The clinical work has been carried out almost entirely by two surgeons and by two British Sisters with the support of a staff nurse seconded by the Director of Medical Services. The number of new cases seen during the year was 21,776, of whom 17,700 were Moslems, 3,270 Christians, and 806 Jews. The total number of patients suffering from acute conjunctivitis was 7,507, and 850 of these were complicated by corneal ulceration, which went on to perforation in 176.

ESTIMATION OF SERUM PROTEINS

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During recent years interest has been focused on the concentration of proteins in serum, as it is possible that, besides the gross reduction that is found in cases of famine oedema, a less severe reduction of the average concentration of protein in serum may occur among a population whose protein intake is low. If such less severe reductions are to be detected, the methods of estimating serum proteins must be such as will give average results which do not deviate from the true values, or values as estimated by some standard method, by at most more than 2%. It appears that the copper sulphate method recently introduced by Phillips, van Slyke, *et al.* (1944, 1945) is accurate enough if proper precautions are taken and the correct formula is used in calculating the protein concentration from the observed specific gravity.

Chibnall, Rees, and Williams (1943) have pointed out that considerable laxity has crept into the technique of estimation of nitrogen by the Kjeldahl method; their main criticism was that the times allowed for digestion of the proteins were not sufficient to ensure the conversion of all the nitrogen to ammonia. Our attention in this matter was aroused when we tried the copper sulphate method of measuring specific gravity. The relation between the specific gravity found (S) and number of grammes in 100 ml. of serum (P) is expressed by Equation 1:

$$P = K\ (S - A),$$

where K and A are constants. Moore and van Slyke (1930) found that concentrations of protein in serum of patients suffering from nephritis could be calculated from the specific gravity found by weighing. The appropriate values for K and A in Equation 1 for such sera were 343 and 1.007. However, the results obtained by Moore and van Slyke with 9 sera from normal persons did not fit this equation. In the first report (1944) of the copper sulphate method the values of K and A appropriate for this method were again given as 343 and 1.007. It was stated by workers in this country that concentrations found by the method, using this formula, agreed with those deduced from nitrogen estimations by the Kjeldahl method. We, however, found that if A was taken as 1.007 the value of K, when the method was used in sera from women before and shortly after delivery, was in the neighbourhood of 380; and in the later edition of their report on this method Phillips *et al.* (1945) give the value as 377 for use with normal sera, and 360 for use with pathological sera. The apparent discrepancies between the results of Kjeldahl estimations led us to reconsider the method that we were using.

Prof. Chibnall kindly had the total nitrogen of 15 sera, on which we also were working, estimated in his laboratory, using the reagents as described in the paper quoted and digesting for 17 hours. On the average, the results obtained by the method which we were then using were approximately 2% too low. We have assumed that the nitrogen found by Prof. Chibnall's technique was 100% of the true value. Table I shows the results obtained on a series of sera with different amounts of compounds of selenium and different times of digestion; in all these we used 0.2 ml. of serum, 0.17 ml. of 30% solution of copper sulphate, and 1 ml. of sulphuric acid; after 10 minutes' digestion 1 g. of sodium sulphate was added. If selenium was used as catalyst 0.1 ml. of sodium selenate solution (0.85 g. per 100 ml.) or an equivalent amount of selenium dioxide was added after cooling. The results are given in Table I.

To test the method most commonly used in clinical laboratories, 10 sera were digested with sulphuric acid and copper sulphate, but only 0.3 to 0.5 g. of sodium sulphate, until straw yellow; 2 or 3 drops of hydrogen peroxide were then added and the mixture boiled for a further 3 minutes. This method